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FULL LENGTH ARTICLE

The study of plant protein accumulation in gut of insect using proteomics technique: Wheat–sunn pest interaction

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Abstract Sunn pest, Eurygaster integriceps, is a serious pest of wheat and barley in Iran. The gut and salivary glands are main parts of digestive system in sunn pest. The performance of these organs in digestion is related to its' expressed proteins. The use of proteomics technique to study plant protein behaviors in gut of insects is new method in insect–plant interaction experiments. In this study, some of plant protein spots were traced in adult gut of sunn pest using 2 DE, mass spectrometry and NCBI database. Six proteins contain serpin, β-amylase, α-amylase inhibitor, dehydrosacorbate reducyase, triticin and α-L arabinofuranidose were identified using plant database. The study of sunn pest–wheat interaction and identification of effective proteins in stability of this relation can be helpful for finding of new target proteins in pest control.

1. Introduction

Phytophagus insects have divided two groups' generalist and specialist that feed on several hosts and one or few host, respectively (Fürstenberg-Hägg et al., 2013). Sunn pest as a main pest of strategic crops (wheat and barley) in Middle East, particularly Iran, was considered as specialist insect (Critchley, 1998). Although many of management tools were used for its suppressing, but chemical control is interested tactic for its control, nowadays.

Hemipterous insects have special approach feeding in the world animals. Extra oral digestion is the first step of hemiptera feeding. In this stage, saliva proteins inject to plant tissue and preliminary digestion was performed (Habibi et al., 2008; Javaheri et al., 2009). After it, digestion was completed in gut of them (Liu et al., 2009). The midgut is key part of digestive system that has longest section in comparison with the other parts of alimentary canal. Gut contains of digestive, defensive and skeletal proteins which expression of them affected by abiotic and biotic factors (Pauchet et al., 2008). The optimal growth of phytophagus insects related to their ability in the utilization of essential molecular in their hosts. Some of the plants used from defensive proteins as disruptors in digestive

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process against their parasite particularly insect pests. Protein inhibitors such a protease inhibitor, amylase inhibitor, and chitinase were reported from difference plants (Jouanian et al., 1998). Plant defensive proteins act against both the secreted and structural proteins in gut of insects. Nowadays, using of anti-insect proteins has been considered as an ideal approach in pest management. In co-evaluation process, there were direct evidences that showed of some plant defensive proteins accumulated in lumen of insect (Saadati et al., 2012b). The role of defensive plant proteins in gut of insect is not clear completely and need to be targeted in the new researches.

There are two categories of defensive compound in plant with insecticidal activity contains non-protein metabolite like alkaloide, terpenoid, rotenoide, tannins, cyanogenic glycosides and protein metabolites like the most of enzyme inhibitors (Gatehouse, 1991). Some of these proteins are constitutive in plant tissues or induced after receiving of the phytophagus signals. The most of these signals are existed in the Insect Oral Secretions (IOS) (Fürstenberg-Hägg et al., 2013). These signals have various effects on the defense system of plants. Some of them elicit and some of them may suppress defensive reactions in the plant tissues (Chen et al., 2007).

Gut and salivary gland proteome of sunn pest were studied by Saadati et al. (2012a, 2012c). About 15 proteins that accumulated in adult and fifth instar nymphs' sunn pest were reported by Saadati et al. (2012b). Every identified proteins were classified in the special groups such as carbohydrate, lipid and protein metabolism, defense system, muscular system (Saadati et al., 2012a, 2012b, 2012c).

One of the main effective factors in normal development of invader insects is quality of hosts. Nowadays, insect–plant interactions are interesting studies to deeply understand co-evolution. However, some of biomolecules that entered to gut of insects can be considered as key index in insect–plant interactions. For example, role of plant defense protein and its effects on gut proteins can be used as new opportunity for using them as spray biopesticides or protein inhibitors expression in transgenic plants.

In our previous studies, proteome of digestive system in adult sunn pest and fifth-instar nymphs were identified (Saadati et al., 2012a, 2012b, 2012c). In this research, some of protein spots in proteome map of gut in recurrent sunn pest after two days feeding were selected to identify. Our purpose was tracing of defensive plant proteins in gut of sunn pest after feeding from wheat.

2. Materials and methods

Recurrent adult insect was collected from wheat farm around Tabriz area in spring 2011. Insects were reared on wheat var. Alvand in 27 °C ± 1 and humidity 40% with 16:8 (L:D) photoperiod regime. Gut of two-day-old adults dissected and washed with PBS. After dissection, guts were transferred to microtube contains ice PBS and cocktail of protease inhibitors.

2.1. Protein extraction

Acetone/trichloroacetic acid method was used to protein extraction. Three guts with one ml were homogenized and centrifuged at 30,000*g*, 30 min, and 4 °C to remove insoluble materials. Gut proteins were precipitated by 10% trichloroacetic acid and then washed by 100% acton three times and pellets were solubilized in lysis buffer (7 M Urea, 2 M thiourea, 2% CHAPS, 60 mM DDT and 1% ampholyte (pH: 3–10)). Insoluble material was removed after two times centrifugation (20,000*g*, 20 min, and 25 °C). Total protein was determined according to Bradford method using protein dye reagent and bovine serum albumin as standard.

2.2. Two-dimensional polyacrylamaide gel electrophoresis (2-DE)

A total of 400 μg of extracted proteins were separated in the first dimension by isoelectric focusing (IEF) tube gels and in the second dimension by SDS–PAGE. An IEF tube gel of 11 cm length and 3 mm diameter was prepared. IEF gel solution consisted of 8 M urea, 3.5% polyacrylamide, 2% NP-40, 2% ampholines (pH 3.5–10), ammonium persulfate and TEMED. Electrophoresis was carried out at 200 V for 30 min, followed by 400 V for 17 h and 600 V for 1 h. After IEF, SDS–PAGE in the second dimension was performed using 15% polyacrylamide gels with 5% stacking gels. The gels were stained with Coomassie brilliant blue (CBB), and the position of individual proteins on gel was evaluated automatically with Melanie 7 software.

2.3. Protein identification

Protein spots excised from CBB-stained 2-DE gels were incubated in 50% acetonitrile and then washed in 50 mM NH4HCO3 for 15 min. Proteins were reduced with 10 mM DTT in 50 mM NH4HCO3 for 20 min and alkylated with 40 mM iodacetamide in 50 mM NH4HCO3 for 15 min, then digested with trypsin at 37 °C. The resulting peptides were concentrated and desalted using a NuTip C-18 pipet tips and then injected into an Ultimate 3000 nano LC coupled to a nanospray LTQ XL Orbitrap MS. After converting Tandem mass spectrum DTA files to MGF files, peptide masses were searched in National Center for Biotechnology information (NCBI) database using Mascot search engine (www.Matrixscience.comq). Search parameters were 0.5 Da for mass tolerance and 10 ppm for peptide mass accuracy. Only one missed trypsin cleavage was allowed and carbamidomethylation of cysteines and oxidation of methionines were selected as fixed and variable modification, respectively.

3. Results and discussion

Two-day-old adults were dissected and crude proteins were extracted from guts. Proteins were separated by 2-DE and visualized by CBB (Fig. 1). 212 spots were detected by using Melanie software that the most of them were identified in our previous studies (Saadati et al., 2012b, 2012c). Eleven spots were selected to identify using Plant and non animal's database with mascot engine. Six plant proteins; Triticin, serpin, α-amylase inhibitor, α-L arabinofuranidose, dehydroscorbate reductase and β-amylase, that match with the corresponding proteins from *Triticium aestivum,* were accumulated in gut of adult sunn pest (Table 1). In our previous data many proteins with animal origin were identified in gut tissue of adult sunn pest using tube gel technique (Saadati et al., 2012c). The most identified proteins in the gut proteome of

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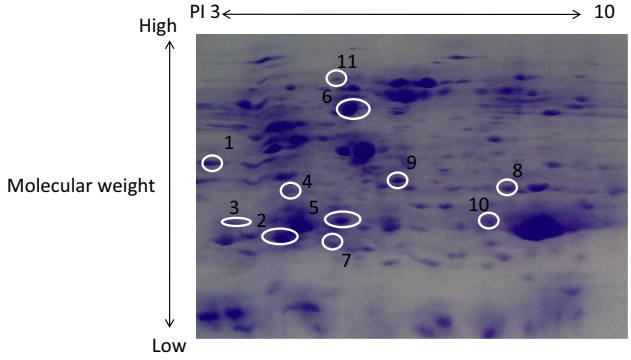

Figure 1 Protein expression patterns in gut of adult sunn pest. One-day-old insects were dissected and proteins were extracted from guts, separated by 2-DE and visualized by CBB staining in adult insects. Circles indicate position of accumulated wheat proteins in guts.

sunn pest were placed in metabolism category (Saadati et al., 2012b, 2012c). Some of digestive proteins (α-amylase, protease, lipase); muscular proteins (actin and tropomyosin), cell metaboloic proteins (glyceraldehide 3 phosphate) and other proteins like HSP70 and catalse were reported from gut of adult and fifth-instar nymphs in sunn pest (Saadati et al., 2012b, 2012c). Role of plant proteins in growth and development of phytophagus insects and plant–insect interaction is scrutiny and new researches are completing our knowledge in these fields. Tree plant proteins contain β-amylase, serpin and dehydroascorbate reductase were reported from gut of adult and fifth-instar nymphs of sunn pest (Saadati et al., 2012b).

Guo et al. (2012) listed various grain proteins that some of them can be used as insecticidal proteins such as α-amylase inhibitors, serpins and chitinases. Proteome pattern of wheat grains contained various proteins that some of them differentially expressed in different developing stages. Wheat grain proteins were classified into prolamins (gliadins and gluteinis) and non-prolamins groups (albumins and glubolins) (from Guo et al., 2012).

Serpins are members of protease inhibitors group that undergo reversible and irreversible conformational changes that cause regulating their activity (Rosenkrands et al., 1994). Existed serpins in wheat and barley contain the highly conserved sequence Pro-Phe-Leu-Phe-Leu which is buried in the interior β-sheet of mammalian serpins. Rosenkrands et al. (1994) suggested that cereals contain numerous low molecular weight inhibitors of digestive enzymes protecting that grain against serious insects. Although serpins were identified in animals world, but relatively little is known about plant serpins biological functions which accumulated in gut of insects (Chen et al., 2007). An important conclusion from this and previous works (Saadati et al., 2012b, 2012c) is that abundant accumulation of serpin in gut of adult sunn pest was not hard barrier against sunn pest enzymes, because amount of feeding in this pest not affected by this inhibitor. Kansal et al. (2008) showed that protease inhibitors when expressed in higher amounts in important crops are effective against phytophagous insects, also after denaturation by cooking, improvement of the quality occurs lead to release of essential amino acids. Extracted serpin from hemolymph of *Panstrongylus megistus* had ideal potential in control of Chagas disease was caused by protozoan, *Trypanosome cruzi* (Moreira et al., 2014).

The gut proteome of phytophagus insects is fighting with plant protease inhibitors by over expressing existed proteinases or producing new isozymes of digestive proteinases to overcome plant defensive compounds (Ussuf et al., 2001). Insect and plant relation is a fight mode, as at the first step insects produced insensitive proteinases in midgut and the other hand for avoiding from this tactic, plants should be stack genes for producing of serpins with different mode of actions (Gatehouse, 1991; Lukasik et al., 2011). In the sunn pest–wheat interaction, it seems that expressed digestive proteinases in the gut sunn pest have overcome to wheat serpins. Protein engineering of the wheat serpins may be can disrupt digestive protease in the next generation of this interaction.

Table 1 The identification of the accumulated plant proteins in gut of adult sunn pest using NCBI (Plant) database.

Spot no.[a]	Description	Acc. no.[b]	Theo. Mr[c]	PI[d]	M.P.[e]	Score[f]	Cov.%[g]
1	Triticin	gi147744620	51.5	5	3	73	12
2	Serpin	gi224589266	43.2	5.6	5	58	10
3	α-amylase inhibitor	gi54778507	13.8	5.7	5	100	34
4	α-L arabinofuranidose	gi18025340	82.9	5.59	19	314	11
5	Dehydroascorbate reductase	gi28192421	23.4	5.88	5	41	16
6	β-amylse	gi32400764	31.1	8.6	10	155	33
7	Not identified						
8	Not identified						
9	Not identified						
10	Not identified						
11	Not identified						

[a] Spot no., the spot number as given in Fig. 1.
[b] Acc. no., accession number according to the NCBI database.
[c] Theo., theoretical; Mr, molecular weight.
[d] Isoelectric point.
[e] M.P., number of query matched peptides, the proteins with more than three matched peptides were included.
[f] Score, ion score of identified protein using NCBI database.
[g] Cov., sequence coverage, the proteins with more than 5% sequence coverage were included.

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α-amylase (EC 3.2.1.1) was reported from gut and salivary glands of sunn pest (Saadati et al., 2008). This enzyme has different isozyme that used in degradation of starch in food materials. The function of α-amylase inhibitor is interfering with insect nutrient utilization like the other enzyme inhibitors (Franco et al., 2002). Bonavides et al. (2007) showed that extracted α-amylase inhibitors from plant seeds can be used as potential inhibitors to interfere with the insect digestion process as alternative tactic in safe pest management. The report of wheat α-amylase inhibitors in gut of sunn pest add a novel piece on sunn pest–wheat interaction puzzle, suggesting that this inhibitor could not affect amount of feeding by depressing of α-amylase activity. The purified α-amylase inhibitor from wheat endosperm had insecticidal activity against various pests like *Calosobruchus maculates*, *Tribolium confusum*, *Sitophilus* sp., *Zabrotes fasciatus* (Gatehouse, 1991). On the other hand it is no efficient against amylase in the Hemiptera and Lepoidoptera (Fürstenberg-Hägg et al., 2013).

Dehydroascorbate reductase (EC1.8.5.1) plays important role in growth of plant cell by regulating of ascorbic acid (Asc) availability (Kato et al., 1997). Asc is main antioxidants that detoxified reactive oxygen species in plant metabolism particularly in chloroplast (Chen and Gallie, 2006). Their results showed that dehydroascorbate reductase in rice is quite different from its enzyme in rat. The insects have different enzymatic and non enzymatic methods to overcome reactive oxygen species such as dehydroascorbate reductase, catalase, and peroxidase (Lukasik et al., 2011; Kato et al., 1997).

Triticin as minor storage protein in wheat endosperm is lysine rich. It recommended adding in wheat flour as supplementary protein due to having lysine rich (Yadav and Singh, 2011). β-amylase (EC 3.2.1.2) is key exoenzyme that catalyzes hydrolysis of the second glycosodic bond in the non-reducing end of the starch molecule. One of the main roles of the most digested plant proteins in gut of adults is releasing of essential amino acids to consume in growth and developing of insects (Jouanian et al., 1998). Many proteins like triticin and β-amylas discovered from gut of insects, but their order of appearance and how they interact with the gut proteome is still unresolved. Although the reasons of more stability of some plant proteins in gut of insect are not clear, but it seems that it is related to insect ability in suppressing of their adverse effects on the normal digestive process.

Plant–insect interaction is interesting opportunity in insect physiology studies. Nowadays, screening for more/new plant proteins as anti-insect compounds and proteome analysis of plant and insect tissues studies are needed to elucidate pathway of sunn pest–wheat interaction.

To our knowledge, this is the first report of accumulation of plant protein in gut of sunn pest. Although many defense components remain to be discovered but our team tried to complete this research in near future.

4. Conclusion

Plant–feeding insects have required to specific physiological changes in proteome of gut to take essential materials for assimilation. The tracing of plant proteins in gut of insects is interesting way for understanding of plant–animal proteins interaction. Using of safe methods in pest management, is a new trend of researches which are focused on the discovery of insecticidal proteins that have no adverse effects on the non target organism particularly humans. The *E. integriceps* was highly specialized for feeding on wheat and other gramineae plants, particularly barley. Reporting of plant protein accumulation in gut of sunn pest can create new opportunity for using of insecticidal proteins in production of transgenic wheat. We hope these results may be helpful in finding of new safe way in sunn pest control and the similar pests. Our research will be continued to identify new insecticidal proteins in gut of sunn pest with original of the other hosts of sunn pest.

Conflict of interest

There is no conflict of interest.

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